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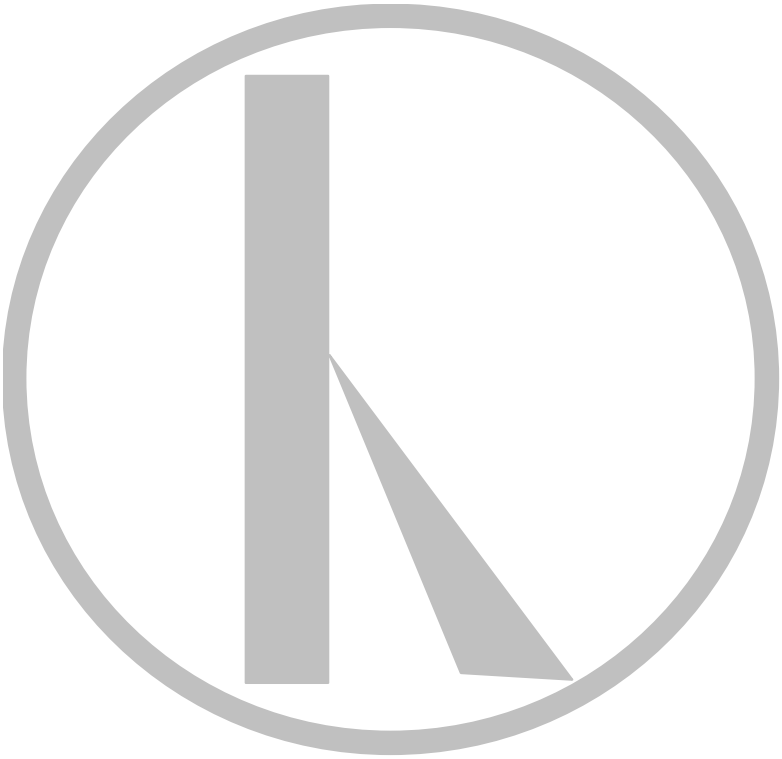

NEWSLETTER OF THE HISTOTECHNOLOGY GROUP of NSW

Contents

Editorial

We are over the hump of 2005 and heading towards Christmas. But before we get there several interesting events are to occur and some milestones have been reached.

A successful annual general meeting was held at ICPMR with a new committee (wearing the same old faces – of capitulation!!) being elected, a thought provoking talk given by Dr James Kench on molecular and immunohistochemical studies being done on pancreatic carcinomas and the awarding of Life Membership to Bill Sinai.

We have our National Meeting in Melbourne on the 21st-23rd October but before this there is a one-day conference in Newcastle entitled *Private and Public Pathology: Crossing the Boundaries*. This is to be held on Saturday, 27th August. Hopefully you will have already received a notice.

This issue includes some Gems from the College of American Pathologists on workload and mucin stains, safety issues with Uranyl nitrate, abstracts from the literature and an innovative PASM stain to try.

Tony Henwood
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Histograph
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MUDGEE

YES!!!!

WE'RE HEADING TO MUDGEE AGAIN! MARCH 16TH, 17TH, 18TH 2006.

IT HAS BEEN FIVE YEARS SINCE OUR LAST SUCCESSFUL MEETING AT MUDGEE AND THE COMMITTEE THOUGHT IT TIME TO REVISIT THIS WONDERFUL VENUE.

WE ALREADY HAVE AN OVERSEAS GUEST SPEAKER WILLING AND ENTHUSIASTIC TO COME AND WE ARE IN THE PROCESS OF INVITING SEVERAL LOCAL SPEAKERS.

THE THEME FOR THE SATURDAY NIGHT WILL BE "ST PATRICK'S DAY"

Chairman's Message

During 2004-2005 the committee has been endeavouring to achieve several goals, one was to give a grant to support the ongoing success of the National Histotechnology Meeting. This grant of $10,000 was sent to the Victorian Histotechnology Group to assist in the preparation for the 2005 National Histotechnology Meeting with the proviso that a the same amount be forwarded to the next state group holding the National Meeting.

During the year several presentations were organized.

1. A group from ACT ran a very successful Immunohistochemistry Workshop which had over 30 participants. This was held at ACT Pathology in Woden Hospital.

2. The Christmas Lecture was at the North Ryde RSL with the guest speaker being …. Who spoke on several topics from the entomological world, including "Maggots in Medicine", "Bed Bugs" and an interesting tale of how Entomology was involved in finding a safe area for horse coming to the Olympic Games.

3. A wet workshop was held at Granville TAFE in November on staining tissue mucins. The day was a success although numbers were small.

4. Other topics for meetings were being discussed for the future.

5. The group has changed the mailing address to make more reasonable access for the secretary.

6. The committee agreed we should have a state weekend get together in 2006. The venue decided on was Mudgee. As all who attended the last Mudgee conference will agree it was a great venue. If anyone has a speaker they might like to hear please let the committee know. We already have an overseas speaker willing to come. The venue has been booked for the weekend of the 17th, 18th and 19th March 2006.

7. A meeting in Newcastle was suggested by Amber Johns and she has begun to organize this and a notice will be circulated shortly. Saturday 27th August 2005.

8. Other meetings proposed for the coming year, as distributed in Histograph include, IHC Retrieval Workshop, Silver stains workshop, Museum Techniques workshop and Cut-up for Technologists.

9. In June we listened to Mr Ted Sheady from Microsearch Foundation about microsurgery to attach limbs to people who had previously lost one or more limbs. A small number were present for this very informative talk.

10. November – December Dr Steven Fairy will talk at the Christmas Lecture being held at the North Ryde RSL a date to be advised.

11. Lastly tonight we have had the privilege of Ass. Prof James Kench to talk to us about “Microarrays and Pancreatic tumours”

12. I would like to thank the committee members for there support throughout another year and wish the new committee all the best for the new year. Also I think we should thank Tony Henwood for his continued success with the Histograph. The new format looks good and makes the newsletter look more professional.

Bill Sinai
Chairman
Histotechnology Group of NSW

Life Membership Awarded to Bill Sinai

In the past there have been several members who have, because of their invaluable service to our discipline, been awarded lifetime achievement awards. Such members as Alan Smith, Lenore Beckenham, Bruce Munro and Garth Maxwell to name a few have been an inspiration to many of us. At the last annual general meeting a motion was passed to change the group’s constitution to include a new class of membership namely the Life Member classification. The motion was as follows:

> "Life membership may be conferred for continued valuable service to the NSW Histotechnology Group of NSW. To be eligible the proposee needs to have been a continuous financial member for a minimum of five (5) years.
> Nominations must be seconded and this distinction shall be conferred only at a General Meeting.
> The distinction shall carry with it the right to attend and to speak at all General Meetings of the Association. They will be entitled to vote at all general meetings.
> Life Membership may only be granted by a THREE QUARTER (¾) majority of Delegates and Life Members present and voting at the Annual General Meeting and shall be by secret ballot.
> A life member shall not be required to pay membership fees or dues for the rest of their life.
> A Life Member's badge shall be awarded."

Following the adoption of this amendment to the constitution, Bill Sinai was nominated and unanimously endorsed as our first Life time member.

The meeting believes that Bill's contribution has had a profound impact on the continued success of the NSW Histotechnology Group over the many years that he has been involved. Bill has been chairman for over ten years and has been the active instigator and organiser of our successful State meetings and our inaugural National Meeting.

Congratulations Bill, a reward well deserved.

Is Uranyl Nitrate A Significant Risk To Histologists?

Ron Hightower
Director, Quality Control Dept.
American Histology Reagent Co., P.O. Box 2539, Lodi, CA 95241.

Several articles appeared in various publications between June and September 1996 regarding the storage, use, and handling of Uranyl Nitrate by histologists.

Uranyl Nitrate in solution was developed by Gilbert Steiner over 70 years ago to allow precise demonstration of spirochetes in single sections of formalin fixed paraffin embedded tissue. Steiner's basic method has been modified by many of us to incorporate the use of a microwave oven to decrease procedure time while maintaining the high stain quality and reproducibility that the traditional method affords.

As these articles circulated through our "community", I began receiving calls from histologists who thought, or were told by their facility safety officer, that they could no longer have solid Uranyl Nitrate in their lab and the 1% Uranyl Nitrate Solution used in our Steiner Stain Kit would have to be stored in a lead container. It appears these articles caused a considerable amount of concern and confusion about a chemical that has been routinely and safely used for many years, so I would like to present some additional information intended to assist you in reaching an informed decision regarding the use of this chemical and solutions containing it.

Uranyl Nitrate is a solid crystalline material derived from uranium, a naturally occurring weakly radioactive material mined from the earth's crust. The amount of uranium contained in commercially available Uranyl Nitrate is so small that this chemical is not regulated by the U.S. Nuclear Regulatory Commission, which is the regulating authority for radioactive materials. While uranium is recognized as a radioactive material by most people, other substances found in nature are also radioactive; "cosmic rays" that enter earth's atmosphere from space, combined with naturally occurring radioactive materials, are responsible for "background" radiation that we receive every day. Terrestrial background radiation is approximately 0.02 millirems per hour (mR/hr); the NRC has established occupational exposure limits of 5,000 millirems (5 Rem) per year whole body exposure and 75,000 millirems (75 Rem) per year for hands, forearms, ankles and feet.

Pregnant workers are limited to 10% of the above limits during gestation. In independent tests performed at American Histology Reagent Company, Inc. by *Kenneth C. Lamson, Certified Health Physicist (CHP), a surface measurement of a glass stock bottle containing 25 grams of Uranyl Nitrate crystals measured 0.4 mR/hr; at a distance of 1 foot the reading was indistinguishable from the natural background level of approximately 0.02 mR/hr.

Measurements of a plastic bottle containing 100 ml of 1% Uranyl Nitrate Solution (a component in our Master*Tech Steiner Stain Kit) were also 0.4 mR/hr at the surface of the bottle and 0.02 mR/hr at 1 foot. Based on the above measurements, a lab technician in surface contact with either of the above containers 8 hours per day, 5 days a week, for 50 weeks would receive an external radiation dose of less than 25% of the allowable annual limit; in typical lab use, actual exposure is probably less than 1%!

Radioactive isotopes are among the most regulated substances that exist and have been extensively studied by the N.R.C. and other federal government agencies. Uranyl Nitrate crystals and stain kits containing 1% Uranyl Nitrate Solution sold by American Histology are exempt from U.S. Nuclear Regulatory Commission and State of California radioactive regulations! Because Uranyl Nitrate is a heavy metal, use of the crystalline form in the histology lab requires protection from ingestion, inhalation, prolonged skin contact, or entry via skin abrasions or cuts. Solutions of Uranyl Nitrate should be used with gloved hands and normal laboratory protective clothing. Plastic backed absorbent material is recommended to protect work areas; if a spill occurs, the absorbent material can be discarded with normal lab refuse. When correct laboratory procedures and good personal hygiene practices are followed, Uranyl Nitrate crystals or low percentage solutions do not present significant health or environmental risks.

Some technicians have reported success when 10% Zinc Formalin is substituted for 1% Uranyl Nitrate in a modified Steiner Stain procedure. Our experience with this technique have been unsatisfactory and it is my opinion that Uranyl Nitrate provides optimum spirochete demonstration when using either Gilbert Steiner's original procedure or the common modifications used in most histology labs.

Lysenin: A new tool for investigating membrane lipid organization

Reiko Ishitsuka & Toshihide Kobayashi
Anatomical Science International 79(4): 184 – 190

Sphingomyelin is a major sphingolipid species in animal cells and is a major lipid constituent of plasma membranes. Recent reports have established important roles for sphingomyelin and its metabolites as second messengers in signal transduction events during development and differentiation. Sphingomyelin is also a major component of sphingolipid, cholesterol-rich plasma membrane microdomains, known as ‘lipid rafts’. However, little is known about the organization of sphingomyelin in biological membranes. Lysenin is a recently discovered sphingomyelin-specific toxin. In the present review, we summarize the current characterization of this protein and describe our recent attempt to elucidate the organization of sphingomyelin in cellular membranes using lysenin as a unique tool

Aspects of oxalosis associated with aspergillosis in pathology specimens

Uğur Pabuççuogˇlu
Pathology - Research and Practice Volume 201(5): 363-368, 2005.

Oxalosis (calcium oxalate deposition) is associated with various conditions, including aspergillosis. Some Aspergillus species produce oxalic acid, which reacts with blood or tissue calcium to precipitate calcium oxalate. Calcium oxalate crystals exhibit various shapes and are strongly birefringent. These occur in cytological specimens, as well as in tissues of patients with Aspergillus infection.

Aspergillus species are hyaline septate moulds, and they can be accurately recognized in pathology specimens only if conidial heads (fruiting heads) are present. When these structures are not observed, detection of associated oxalosis in a mould infection supports the pathological diagnosis of aspergillosis.

The presence of oxalosis is helpful when microbiological identification or immunohistological techniques for fungi are not available. Calcium oxalate crystals can induce cellular injury by several mechanisms, and there is increasing evidence that oxalosis-induced tissue damage may occasionally lead to a poor clinical outcome.

This review discusses the diagnostic value and the potential clinical significance of oxalosis associated with aspergillosis.

Histomathematical Analysis of Clinical Specimens: Challenges and Progress

Gallya Gannot, John W. Gillespie, Rodrigo F. Chuaqui, Michael A. Tangrea, W. Marston Linehan and Michael R. Emmert-Buck,
Journal of Histochemistry and Cytochemistry 53(2): 177-185, 2005

Proteomic analysis of clinical tissue specimens is a difficult undertaking. Described here is a multiplex study of protein expression levels in histological sections of human prostate that addresses many of the associated challenges. Whole-mount sections from 10 prostatectomy specimens were studied using 15 antibodies, immunohistochemical staining, digital imaging, and mathematical analysis of the data sets. The approach was successful in stratifying cell lineages present in the samples based on proteomic patterns, including differentiating normal epithelium from cancer. This strategy likely will be a useful method for extending the number of proteins that can be analysed in clinical cancer specimens using currently available laboratory techniques.

Juvenile cirrhosis in a 16th century Italian mummy. Current technologies in pathology and ancient human tissues

R. Ciranni and G. Fornaciari
Virchows Archiv 445(6): 647-650, 2004

The natural headless mummy of a young man from the Basilica of Saint Domenico Maggiore in Naples (16th century) showed at autopsy a well-preserved fibrous liver with a nodular surface, suggesting a case of cirrhosis. Stereo and light microscope study confirmed this diagnosis. To identify the possible aetiology of this cirrhosis, additional techniques currently used in pathology were performed.

Hemochromatosis and alpha1-antitrypsin deficiency were investigated without results. Investigation regarding Wilson's disease gave positive results, since the use of rhodamine staining, which is specific to detect the presence of copper in tissues, resulted in red-brown grains at light microscopy.

The positive rhodamine test was invalidated by atomic absorption spectroscopy (AAS), which revealed normal copper levels in the tissues. These negative results and the clear and diffuse macronodularity of the liver suggest a case of post-necrotic cirrhosis.

Mechanisms of Heat-induced Antigen Retrieval: Analyses In Vitro Employing SDS-PAGE and Immunohistochemistry

Shuji Yamashita and Yasunori Okada
J Histochem Cytochem 53:13–21, 2005.

In this study, we examined the mechanism of heat-induced antigen retrieval using analytical procedures involving SDS-PAGE, Western blotting, and immunohistochemistry. Five proteins were treated with 4% formaldehyde in the presence or absence of 25 mM CaCl2, then heated under various conditions after removal of formaldehyde and analyzed on SDS-PAGE. Formaldehyde produced inter- and intramolecular cross-links in the proteins. Heating at high temperatures cleaved these cross-links at all pH ranges examined (pH 3.0, 6.0, 7.5, 9.0) and produced almost the same electrophoregrams as the native proteins. Proteins treated with formaldehyde containing CaCl2 showed similar electrophoretic patterns, observed without heating or after heating at pH 6.0 and pH 9.0 in the presence or absence of 10 mM EDTA. Western blot analyses demonstrated that the soluble forms of ß-actin (monomer and oligomers) and fibronectin were present in extracts from deparaffinized mouse uterine sections autoclaved for 15 min but not in extracts from unheated specimens. Nine of ten antigens, independent of their isoelectric points, exhibited much stronger immunoreaction in the sections heated at pH 9.0 than in those heated at pH 6.0. The second heating at pH 6.0 significantly decreased the immunostaining of the antigens that had been boiled at pH 9.0, but the immunostaining was recovered after a third heating at pH 9.0. These results suggest that the main mechanism of heat-induced antigen retrieval is disruption of the cross-links and that pH is an essential factor for a proper refolding of epitopes.

Typing of Mucins by Histochemistry

The following question and answer was posted on the CAP website (http://www.cap.org/apps/docs/cap_today/q_and_a/qa_0302.html).

Q. Besides its role in diagnosing mesotheliomas, does the typing of mucins by histochemistry (neutral versus acidic, sulfo- versus sialo-) have practical applications in neoplastic surgical pathology?

A. The current role of mucin histochemistry in the differential diagnosis of neoplasms is limited, as this function has been assumed in large part by diagnostic immunohistochemistry. Potential applications of mucin histochemistry include but are not limited to:

Differentiating stromal and epithelial mucins. Stromal mucin is positive with the colloidal iron (CI) and Alcian blue (AB) stains and is removed by hyaluronidase digestion. Stromal mucin is typically negative with the periodic acid-Schiff stain following diastase digestion (PAS-D) and minimally mucicarmine positive.

In contrast, epithelial mucins are PAS-D and/or mucicarmine positive and, when positive with the CI and AB stains, are hyaluronidase resistant. This application is still used, albeit less frequently today, when attempting to distinguish carcinoma from epithelial mesothelioma. This technique can be applied also as an adjunct to keratin immunohistochemistry in distinguishing carcinoma, particularly sarcomatoid forms, and synovial sarcoma from other sarcomas with epithelioid features; the latter exhibit only stromal mucin

Identifying glandular differentiation in adenocarcinoma. Documenting of intracytoplasmic mucin is a rapid and cost-effective method for establishing a diagnosis of adenocarcinoma. It is important to remember, however, that epithelial neoplasms do not reproducibly produce acid or neutral mucins. Therefore, identifying epithelial mucin requires using two stains, singly or in combination, typically PAS-D (neutral) and Alcian blue or mucicarmine (acid).

Differentiating extraskeletal myxoid chondrosarcoma and chordoma (Alcian blue and colloidal iron positive, hyaluronidase resistant) from other myxoid neoplasms, particularly myxoid liposarcoma (AB/CI positive, hyaluronidase sensitive).

Differentiating chromophobe renal cell carcinoma from conventional renal cell carcinoma and oncocytoma. This more recent, technically difficult application requires use of the Hale's technique with careful attention to pH. Chromophobe renal cell carcinoma will show a diffuse strong reaction with a characteristic reticular pattern; the other neoplasms are characteristically negative or focally positive with a granular or droplet-like pattern.

Identifying high-risk gastric intestinal metaplasia (IM). Incomplete intestinal metaplasia (types II and III) can be further subtyped using the high iron diamine-Alcian blue (HID-AB)

stain to identify subsets of acid mucins. The HID-AB stains sialomucins blue and sulfomucins brown-black. Using this stain, type III IM, in which the columnar epithelial cells contain sulfomucins, can be differentiated from type II IM, in which the columnar cells contain only blue-stained sialomucins.

Some, but not all, studies have shown that type III is the form of IM seen most frequently in association with adenocarcinoma; therefore, identifying this form may warrant close follow-up, additional biopsies, or both. Recently, the presence of sulfomucins has been associated with progression to adenocarcinoma in Barrett's esophagus.

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Weiss SW, Goldblum JR. Enzinger and Weiss's Soft Tissue Tumors. 4th ed. St. Louis, Mo.: C.V. Mosby; 2001.

How many cassettes should be processed by a histotechnologist during an eight-hour shift?

This question and answer was posted on the CAP website (http://www.cap.org/apps/docs/cap_today/q_and_a/qa_1102.html). What are your thoughts

No established comprehensive standard addresses histology workload. Previously published CAP workload guidelines for histopathology, based on data from the Laboratory Management Index Program, say "each well-trained HT/ HTL can be expected to produce approximately 3,000 slides per quarter, or 12,000 slides per year. Included in these totals are 2,500 H&E slides and 500 common special stains, or 10,000 H&E slides and 2,000 common special stains. If only rare special stains are requested, more set-up time is required. Twelve thousand slides per year is equivalent to 50 slides per day. These are average numbers for a lab where much of the work is not automated."

In practice, a uniform standard across laboratories may be an unrealistic goal because many factors influence the number of blocks a histotechnician/ technologist can cut in a given period. These include:

- The experience level of the technician. A new employee or student would be expected to cut at a slower rate.
- The case complexity. Biopsies, which require multiple levels and careful trimming, require considerably more time than routine cases (for example, uterus).
- The number of interruptions. Smaller laboratories, in which the cutting technician may be answering the phone or receiving special stain or recut requests, or both, will have lower productivity.

The most useful standard for employees in a given laboratory is set by the supervisor or senior technologists, or both, based on past productivity levels.

To address productivity in a more global sense, it is necessary to assign work units to each of the varied tasks in histology, including loading and maintaining processors, embedding, cutting, routine staining, special stains, and immunohistochemistry. It is then possible to benchmark units worked per hour. As with routine cutting, however, the assignment of work unit values to a given task can only be done realistically by the histology supervisor and pathologist at a given site, taking into account economies of scale and levels of automation.

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A Stain to Try - PASM

The following modification has been used in the Histopathology Department at the Children's Hospital at Westmead for a while now and we are very excited by it!!

This technique demonstrates basement membranes in thin sections. It is especially useful in the study of renal disease. The classical technique involves the treatment of thin 2µm sections with periodic acid followed by heated methenamine silver (Gladwin 1971, Hinds et al 1987).

Non-specific staining can occur resulting in stained nuclei and connective tissue. In the kidney, over-staining of the mesangial matrix can be troublesome. Hayashi et al (1989) have modified this procedure by including a treatment with thiosemicarbazide before silver impregnation. This enhances the deposition of silver, decreasing background staining.

It is believed that the hydrazine group ($-NHNH_2$) of thiosemicarbazide blocks the aldehyde group produced following periodic aid treatment and the thiocarbamyl group ($-SCNH_2$) of thiosemicarbazide reduces the methenamine silver.

Reagents:

1. 1% Periodic Acid

2. 0.5% aqueous Thiosemicarbazide

3. Stock Methenamine Silver

10% aqueous silver nitrate	10 ml.
3% hexamine	400 ml.
Distilled water	10 ml.

Store in a brown bottle at 4^oC

4. Methenamine Silver Working solution

5% aqueous sodium tetraborate	5 ml.
Stock Methenamine solution	50 ml

5. 0.1% Gold chloride

6. 2% Light Green - Warning: Suspected Carcinogen – see MSDS

Procedure:

1. Dewax and hydrate sections

2. Place in 1% periodic acid, 10 min
3. Wash slides in water
4. Rinse in distilled water
5. Place in 0.5% aqueous thiosemicarbazide , 5 min
6. Wash in water, 5 min
7. Rinse in distilled water
8. Place in Methenamine-Silver Working Solution at 60^{o}C. Check slides after 15 minutes. Basement and capillary membranes should be stained up sufficiently after about 20 minutes. The glomerular capsule should be black and the capillary loops dark brown.
9. Rinse in Distilled water
10. Tone in 0.1% Gold chloride 2 min
11. Rinse in water
12. Counterstain in 2% Light Green 20 seconds.
13. Wash quickly in water
14. Dehydrate, clear and coverslip

Results:

Basement membranes	Black
Collagen	Green

Notes:
If over-stained with silver, then a dilute solution of potassium ferricyanide can be used as a reducer.

References

Gladwin (1971) J.Clin.Path. 24:699.
Hayashi et al (1989) Stain Technol 64(4):185-190.
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Proposed Future Events.

Date	Presenter	Where	Topic
27th August 2005	Several	Mayne Pathology Newcastle	The difference between Public and Private Pathology!
September 2005	Andrew Kennedy and Grant Taggart	Mayne Pathology	Cut-Up for Histotechnologists.
November 2005	Andrew Kennedy	Unknown	Museum Techniques
December 2005	Dr Steven Fairy	North Ryde RSL	Renal Tumours
To be advised	Histotechnology Group of NSW	Granville TAFE Lab C264	Microtomy Workshop
To be advised	Histotechnology Group of NSW	Granville TAFE Lab C264	Silver Stains
17-19th March 2006	Histotechnology Group of NSW	Country Comfort Motel Mudgee	Many Speakers

If you have a particular topic you would like to see or know someone you think might be interesting to hear, contact the committee and we will endeavour to present the topic or speaker. The group needs your support, also as it is now compulsory for laboratory staff to attend educational sessions as part of NATA/RCPA accreditation, these sessions are invaluable as evidence of this continuing education. The NSW Histotechnology Group will issue certificates of attendance for all meetings.

The committee Histotechnology Group of NSW.

HISTOTECHNOLOGY GROUP of NSW

ABN: 63 128 868 343

nswhistogroup@bigpond.com

2005 - 2006

I wish to become a member of the Histotechnology Group of N.S.W. and enclose

PLEASE TICK

- ☐ $38.50 for annual subscription of $35.00 and $3.50 GST.
- ☐ $16.50 for student subscription of $15.00 and $1.50 GST (Full-time or working toward first qualification)
- ☐ $82.50 for company subscription of $75.00 and $7.50 GST (2 representatives, one of whom must be a NSW representative)
- ☐ RENEWALS ☐ ANY CHANGES TO PREVIOUS DETAILS.

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